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Role of MicroRNA in Endothelial Dysfunction and Hypertension

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Abstract
Purpose of Review Hypertension is either a cause or a consequence of the endothelial dysfunction and a major risk factor for cardiovascular disease (CVD). In vitro and in vivo studies established that microRNAs (miRNAs) are decisive for endothelial cell gene expression and function in various pathological conditions associated with CVD.

This review provides an overview of the miRNA role in controlling the key connections between endothelial dysfunction and hypertension.

Recent Findings Herein we summarize the present understanding of mechanisms underlying hypertension and its associated endothelial dysfunction as well as the miRNA role in endothelial cells with accent on the modulation of renin-angiotensin-aldosterone-system, nitric oxide, oxidative stress and on the control of vascular inflammation and angiogenesis in relation to endothelial dysfunction in hypertension. In particular, latest insights in the identification of endothelial-specific microRNAs and their targets are added to the understanding of miRNA significance in hypertension.

Summary This comprehensive knowledge of the role of miRNAs in endothelial dysfunction and hypertension and of molecular mechanisms proposed for miRNA actions may offer novel diagnostic biomarkers and therapeutic targets for controlling hypertension-associated endothelial dysfunction and other cardiovascular complications.

Keywords Hypertension · Endothelial dysfunction · MicroRNAs

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Introduction

Hypertension or high blood pressure represents one of the most common complex disorders, affecting approximately 40 % of the world adult population, and 51 % of deaths result from coronary artery disease and cerebrovascular disease [1]. World health statistics 2012 has estimated the prevalence of hypertension to be 29.2 % in males and 24.8 % in females and this is projected to increase to 1.5 billion worldwide by the year 2025 [1]. The prevalence of hypertension enhances with advancing age, so that approximately 90 % of people who are non-hypertensive at 55 or 65 years will develop hypertension by the age of 80–85 [2]. This disease affects countries across all income groups, but the risk of death is higher in low- and middle-income countries. In the industrialized countries, only 7 % of deaths caused by high blood pressure occur under age 60, whereas in the African Region this increases to 25 % [2]. Moreover, the epidemiological study of hypertension has indicated that morbidity and mortality associated with hypertension is going to become a principal health challenge in the twenty-first century [2].

In this context, it is important to point out that the main characteristics of hypertensive patients are: (1) the endothelial dysfunction caused by renal vasoconstriction induced by the elevated uric acid levels; (2) augmented activity of the sympathetic nervous system with increased release of, and

amplified sensitivity to, catecholamines. These lead to further activation of the renin-angiotensin-aldosterone-system (RAAS), not only in kidney but also in other areas. The level of renin, the same as salt and water retention, is improperly elevated in most patients with chronic kidney disease (CKD). In these patients, afferent impulses from the diseased kidney and leptin accumulation have been suggested as possible causes for increasing the activity of sympathetic nervous system that also contributes to hypertension. Furthermore, the endothelial dysfunction and chronic hyperparathyroidism that enhance the sensitivity to calcium and catecholamines participate to hypertension observed in CKD. In addition to mentioned factors, hypertension is associated with endocrinopathies like hyperthyroidism, hyperparathyroidism, Cushing syndrome, Conn syndrome, and phaeochromocytoma [3].

The discovery of microRNAs (miRNAs) has opened new avenues for studying and understanding hypertension and hypertension-associated endothelial dysfunction, featuring a post-genomic era of biomedical research. These non-coding regulatory RNA molecules of ~22 nucleotides have emerged as potential biomarkers, effectors, and targets for diagnosis, prognosis, and therapy in hypertension. Lately, many studies have been done on understanding miRNA biology and function. It seems that miRNAs play an important role in the regulation of almost every cellular process. Usually, a single miRNA can interact with hundreds of mRNA molecules and a specific mRNA molecule may be the target of multiple miRNAs. Thus, miRNA-mRNA interactions may delineate the complex regulatory networks with consequence on the target gene expression and hence on the some biological process. Additionally, the disruptions of miRNA regulation are frequently associated with some pathological states including hypertension-associated endothelial dysfunction.

Endothelial Dysfunction and Hypertension

High blood pressure is the result of elevation in either cardiac output or peripheral vascular resistance, or both. While, the cardiac output is determined by stroke volume and heart rate, the peripheral resistance is controlled by smooth muscle cells (SMCs) from small arteries and arterioles [3] (Fig. 1). A fall in the blood pressure leads to stimulation of the sympathetic nervous system and release of the adrenal medullary hormones: adrenaline and noradrenaline. These lead to augmented cardiac contractility and hence amplified cardiac output and peripheral vascular resistance, in this way increasing blood pressure. An increase in blood pressure causes enhanced vagal tone, leading to bradycardia and vasodilatation [3].

On the other hand, elevated blood pressure results from varying factors such as diet, genetics, race, lifestyle, size (being overweight or obese), and combinations of these [2]. Hypertension, known as high blood pressure, may increase the morbidity and mortality due to other diseases in the presence of other risk factors. For example, in obesity the pathogenesis of hypertension is complex involving hyperinsulinemia and hyperleptinemia.

However, it is generally known that hypertension contributes significantly to cardiovascular disease (CVD) and increases the rate of CVD mortality and morbidity. Furthermore, the hypertension is strongly associated with endothelial dysfunction, a phenotypical alteration of the vascular endothelium that precedes the development of adverse cardiovascular events and prefigures a potential cardiovascular risk [4]. Recent data also emphasize that alterations of the endothelial phenotype constitute a pathogenic risk factor for several vascular diseases including atherosclerosis and future cardiovascular events, but their role in hypertension less is known [5••]. The evidence of an association between hypertension and endothelial dysfunction is substantial and current data suggest that they reinforce each other.

The vascular endothelium represents a dynamic cellular interface between circulating blood and underlying tissue, acting as an integrator and transducer of the humoral and mechanical stimuli [5••]. Thus, the vascular endothelium releases two vasoconstrictor factors, such as endothelin 1 (ET-1) and thromboxane A2 (TXA2), as well mediators of vasodilation, such as nitric oxide (NO), prostacyclin, and the endothelium-derived hyperpolarization factors (EDHF) [6]. A healthy endothelium responds to stimuli continuously releasing potent vasodilators, which have the potential to directly reduce the vascular resistance.

The endothelial dysfunction is defined as the imbalance between the production and bioavailability of endothelium-derived relaxing factors and endothelium-derived contractile factors. As we mentioned above, the endothelial dysfunction is the hallmark of hypertension. The endothelial function can be measurable in a reproducible, valid, and noninvasive manner in hypertensive patients. In hypertensive patients, the vascular endothelial dysfunction has been associated with the reduction of NO release and uninhibited ET-1 mediated vasoconstriction. Also, Widlansky and Harrison in their studies have shown that increased vascular oxidative stress and vascular inflammation are principal characteristics of endothelial dysfunction, and are as well, implicated in the pathogenesis of hypertension [7, 8].

As it is already known, the systemic blood pressure control is ensured by metabolic and local nervous factors, and also by the renal and central control of blood pressure which over-rule local vascular factors. However, the systemic blood pressure control is usually preserved in conditions associated with endothelial dysfunction, such as hypercholesterolemia, even if these may finally result in hypertension development. In particular, some studies have shown that the normalization of

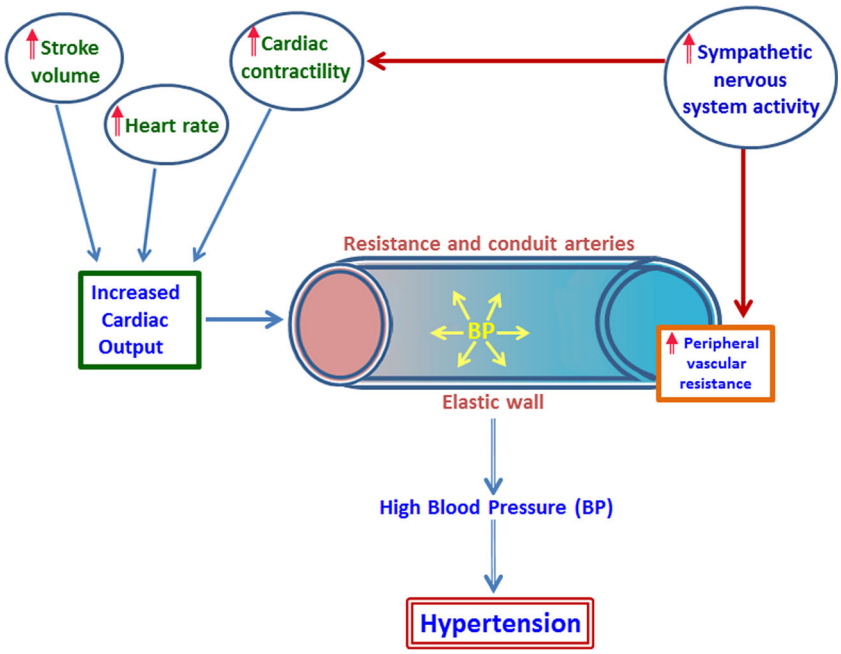

Fig. 1 A suggested cascade of the hypertension development: the blood pressure relationship to cardiac output and peripheral vascular resistance

endothelial function does not seem to affect the blood pressure. On the contrary, in our previous study, we have demonstrated that the administration of an angiotensin II type 1 receptor (AT1R) blocker (irbesartan) has reduced the diastolic and systolic blood pressure and heart rate and had a vasorelaxant effect, improving the endothelial function to simultaneously hypertensive-hyperlipidemic hamsters [9]. These data suggest that the beneficial effect of the irbesartan on endothelial function is not independent of blood pressure lowering. In addition, this effect has been correlated with level decreasing of microparticles (MPs) and level increasing of endothelial progenitor cells (EPCs) in peripheral blood [9]. Also, in the experimental hypertension associated with hypercholesterolemia, the transplantation with EPCs has significantly diminished the systolic and diastolic blood pressure and restored the vascular wall function, increasing the endothelium-dependent vasodilation at acetylcholine [10].

Overall, to establish the relationships between endothelial dysfunction and hypertension, it is imperative to get to know and understand the involved mechanisms. Thus, several studies indicate the contribution of RAAS components, NO release, and reactive oxygen species (ROS) production to the inflammatory and angiogenic responses of vascular endothelium in hypertension and thereafter, to associated endothelial dysfunction.

Renin-Angiotensin-Aldosterone-System and Endothelial Dysfunction

The RAAS regulates a variety of physiological functions, such as hemodynamic equilibrium, electrolyte balance, and circulating volume [11]. Overactivation of the RAAS is central to the pathogenesis of hypertension. As a hormone system, RAAS contains several enzymes, peptides, and receptors [12]. The RAAS cascade begins with the release of renin by the juxtaglomerular apparatus of the kidney into circulation. Although the major source of renin is the kidney, the RAAS is widespread in the body. The RAAS cascade is activated when renin converts angiotensinogen to angiotensin I (Ang I), a weak bioactive decapeptide. Angiotensin-converting enzyme (ACE) removes two amino acids from Ang I generating Ang II, the main effector of RAAS. High Ang II concentrations suppress the renin secretion via a negative feedback loop. Thus, Ang II regulates the blood pressure, aldosterone release by the adrenal cortex, renal sodium and water reabsorption, and vasopressin secretion, and participates in cardiac and vascular remodeling. It also stimulates the release of prostacyclin and catecholamines [13]. Since Ang II is known as principal biological effector, it seems to play a significant role in raising blood pressure as well. In addition to its important role in hypertension, the RAAS has another role in mediating vascular remodeling in neointimal hyperplasia after angioplasty and atherosclerosis [14–16]. The Ang II is to induce vascular remodeling due to its effects on vascular SMC proliferation and hypertrophy. The Ang II growth effects, proliferation versus hypertrophy, are dependent on cell type and cell cycle regulated genes [17]. The vasoconstrictive, proliferative, and pro-inflammatory effects of Ang II are exerted mainly through AT_1R on endothelial cells (ECs) and SMC. In addition, Ang II can bind to the angiotensin II type 2 receptor (AT_2R), considered to be part of the protective arm of the RAAS that has opposite effects to those of AT_1R. Alternatively, Ang II can be catabolized by ACE2 into Ang 1-7, another active peptide of RAAS, which exhibits vasodilator and antiproliferative

properties, acting on the Mas receptor and counterbalancing the actions of ACE/Ang II/AT_1R signaling. In addition, ACE2 can cleave one amino acid from Ang I yielding Ang 1-9. Also, Ang II can be degraded by aminopeptidases to form Ang III (Ang 2-8) and Ang IV (Ang 3-8).

Overall, RAAS through its physiological effectors has a key role in promoting oxidative stress, vascular inflammation, and endothelial dysfunction [17].

Reactive Oxygen Species Production and Nitric Oxide Release Influencing Endothelial Dysfunction

Emerging data implicate multiple sources of oxidative stress in the pathogenesis of hypertension-related endothelial dysfunction [18]. It is well established that an important ROS source is nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) activated by Ang II [19]. The signaling pathway from Ang II to NOX induction and oxidative stress is supported by several mechanisms. One of these involves the cytochrome P450 1B1 (Cyp1B1) in the kidney, which has action mediated in part by the estrogen metabolism [20]. Additional sources for ROS production have been indicated, such as follows: (i) the cyclooxygenase 2, which exhibits an increased expression in small arteries isolated from subcutaneous biopsies of patients with hypertension [21]; (ii) the mitochondrial monoamine oxidase-A and mitochondrial monoamine oxidase-B, which are induced in the mouse vessels and generate the hydrogen peroxide (H_2O_2) sufficient to reduce the endothelial NO release [22]; (iii) p66Shc [23].

In the vascular ECs, the NADPH oxidase represents the main source of ROS. Recent data delineate that the endothelial dysfunction is characterized by increase of the ROS bioavailability and decrease of the antioxidant capacity characterized as oxidative stress as well [6].

Also, increased production of the ROS has been correlated with the vascular NO bioavailability impairment that induces reduced endothelium-dependent relaxation. Many studies highlight the key role of NO of central regulator of vascular endothelial function and associate the loss of NO bioavailability with the occurrence of endothelial dysfunction. This important vasorelaxant factor, NO, is produced by endothelial NO synthases (eNOS) that has activity regulated by substrate, cofactor availability, and electron transfer rate. The ROS generation can affect the regulating factors such as arginine [24] and tetrahydrobiopterin [25] leading to dysfunctional eNOS. Besides, ROS consumption leads to peroxynitrite ($ONOO^-$) and H_2O_2 formation, lower eNOS protein expression, or lack of substrate or cofactor for eNOS [26, 27].

In the mesenteric arteries of spontaneously hypertensive rats (SHR) have been found lower NO and higher H_2O_2 concentrations compared to arteries explanted from control rats [28]. In addition, it has been shown that in hypertension the eNOS could be uncoupled [29], and increased activity of arginase in ECs of coronary arteries impairs the NO-mediated dilation [30]. Moreover, in hypertension associated with hypercholesterolemia, the protein expression of eNOS has been found to be down-regulated, and endothelium-dependent relaxation to acetylcholine reduced into mesenteric resistance arteries [9]. Also, the exposure of carotid arteries isolated from mice to increasing intraluminal pressure has induced the concomitant decreases in endothelium-dependent vasodilation to acetylcholine, rises in vascular superoxide production and NOX activity [31]. Other studies using small interfering RNA (siRNA) and suppression of Rac-1 activity implicate overexpression of integrin-kinase 1 as a key first step in the mechanotransduction of hypertension-induced vascular superoxide production through NADPH oxidase [4].

Inflammation and Endothelial Dysfunction

Alongside oxidative stress, the vascular inflammation is an important characteristic of phenotypical endothelial dysfunction, and its reduction has been shown to reverse endothelial dysfunction [32]. The endothelial dysfunction has been described as a condition including not only the decrease of endothelium-dependent vasodilation but also the inflammatory activation of endothelium [33]. Inflammatory factors such as cell adhesion molecules (CAM), tumor necrosis factor alpha (TNFα), interleukin-6 (IL-6), and C-reactive protein (CRP) have major roles in mediating vascular inflammation. Anyway, some of these inflammatory markers have been clinically associated with CVD events. Interestingly, it has been demonstrated that blocking RAAS negatively modulates the levels of these inflammatory molecules [17].

Other several studies suggest that perivascular adipose tissue and inflammation within adipose tissue play important roles in regulating local and systemic vascular homeostasis. Thus, it has been shown that the adipose tissue from hypertensive rats applied to thoracic aorta segments failed to suppress phenylephrine-induced vasoconstriction, compared to adipose tissue from normotensive animals [34]. Similarly, obese and hypertensive rats with perivascular inflammation have exhibited endothelial dysfunction [35], and obese patients with inflammation in visceral fat depots have also revealed impaired endothelial function [36]. The mechanisms by which the perivascular fat regulates the endothelial function in hypertension, as well as the relative impact on endothelial function in humans of perivascular adipose tissue inflammation are little understood, and future investigations are needed.

The endothelial function under hypertensive conditions is regulated also by the elements of both innate and adaptive immune responses. It has been shown that the activation of innate immunity complement pathway may have negatively impact on the vascular endothelial function [37], whereas

increased anti-inflammatory IL-10 expression from the adaptive immune response reduces the adverse effects on endothelial function in Ang II-associated hypertension [38].

In the experimental hypertension associated with hypercholesterolemia, the endothelial dysfunction has been associated with the increase of pro-inflammatory cytokines [39], and in patients with hypertension and dyslipidemia with altered balance between the levels of endothelial regenerative cells like EPCs and markers of endothelial injury like circulating MPs [40].

All these data indicate that hypertension-associated endothelial dysfunction is closely linked to local vascular inflammation as well as to systemic inflammation.

Defective Angiogenesis and Endothelial Dysfunction

Angiogenesis is the principal mechanism of vascular remodeling in late development and during physiological processes of organ growth and repair [41]. Perturbations of angiogenesis occur in a lot of different pathological states including cancer, diabetes, ischemia, and inflammation. Angiogenesis, known also as developing of new capillaries from existing vascular beds, is a complex process regulated by a controlled balance of proangiogenic and antiangiogenic factors. Disturbance of this balance generates multiple metastatic, ischemic, inflammatory, and immune disorders [42].

Vascular endothelial growth factors (VEGFs), in particular VEGF-A, are implicated in the regulation of the processes required for angiogenesis such as endothelial cell activation, proliferation, migration, and tubule formation [43]. The tyrosine kinase receptor VEGFR2 (flk-1/KDR) is the main receptor responsible for the biochemical effects of VEGF-A on cells and is important for normal vascular development [44]. The activation of VEGFR2 conducts to recruitment and activation of numerous signaling molecules. Among these, there are two mitogen-activated protein kinases (MAPK): p42/44 extracellular signal-regulated kinase 1/2 (ERK1/2) involved in the regulation of endothelial proliferation and p38 MAPK, one of the key modulators of actin cytoskeleton remodeling required for migration [45].

Hypertension may be also an effect of structural and functional alterations of the microvascular network growth resulting in part from disorders in the regulation VEGF, one of the angiogenic factors mostly known. The connection between hypertension, defective angiogenesis, and endothelial dysfunction is convincing. With respect to this, recent investigations have established that ECs are essential players in the expansion, preservation, and remodeling of vascular networks, maintaining in this way the vascular integrity, angiogenesis, and wound repair [46, 47], and VEGF besides its role in the migration and proliferation contribute to maintenance and protection of ECs. In hypertension, an endothelium-mediated pathogenic aspect is the loss of the function of microvessels and defective angiogenesis in organs. The impairment of angiogenesis can contribute to increased peripheral resistance and raised blood pressure and thus to hypertension. The disruption of the balance between proangiogenic and antiangiogenic factors has been found in hypertensive patients [48].

MicroRNAs and Endothelial Dysfunction in Relation to Hypertension

MicroRNA Biogenesis and Function

miRNAs, small non-coding RNA molecules, are post-transcriptional regulators of gene expression by their action on target mRNAs controlling their translation and degradation, modulating in this way various cellular and developmental processes. In brief, two of the RNase III enzymes, Droshua and Dicer, support the formation of small non-coding miRNAs (≈22 nt) from their own genes or from introns. In a first step, RNA polymerase II (Pol II) transcribes miRNA genes into ample polyadenilated RNA molecules, called primary miRNAs (pri-miRNAs) [49]. Primary transcripts with hairpin-shaped loop structure are subsequently endonucleolytic cleaved in nucleus by RNase III enzyme DROSHA associated with DiGeorge syndrome critical region 8 (DGCR8) and other cofactors. New born hairpin-shaped double-stranded precursors (pre-miRNAs) with ≈70 nt in length are next exported into the cytoplasm by exportin-5 (XPO5) and Ran-GTP action. Then, RNase III enzyme DICER and protein TRBP crop the loops of pre-miRNAs, yielding ≈22 nt imperfect miRNA duplexes (miRNA-miRNA*). These double-stranded products consist of functional mature miRNA guide strand and the passenger miRNA* [50]. Only one strand of the complex is in the end incorporated into the RNA-induced silencing complex (RISC), while the other one is being degraded by key integrant parts of the complex, called Argonaut (AGO) protein family [51]. After binding mature miRNA strand, AGO recognizes a specific sequence most commonly located in 3′untraslated regions (UTRs) of targeted mRNA, therefore facilitating miRNA-mRNA imperfect association. This kind of imperfect base pairing endorses the possibility for a single miRNA to target numerous mRNAs. The interaction of miRNAs with its target mRNAs leads to suppression of protein expression mediated by mRNA degradation [52]. Even though the fate of a new miRNA depends mostly by its genomic location, miRNA biogenesis can be regulated at multiple steps by several factors. It was just shown that clustering is the key event of miRNA survival and conservation. Evolutionary conserved miRNAs are significantly enriched in clusters. In this case, new miRNAs are created developing functions related to the pre-existing miRNAs by de novo formation and duplication, two essential mechanisms in miRNA

maintenance [53]. Since miRNA function are directly associated with his structure, analyzing the regulation of miRNA biogenesis provides new insights in the study of miRNAs.

MicroRNAs in Endothelial Cell Biology

The vascular endothelium is constantly exposed to hemodynamic forces, such as the stretch and shear stress resulting from circulatory pressure and flow that are critically involved in maintaining vascular homeostasis. These physical forces that highly regulate endothelial phenotype and function are sensed and translated by ECs into various biological responses through a complex process implying several key molecules, including integrins, G proteins, protein kinases, and miRNAs [54, 55]. By integrating hemodynamic with other biochemical cues, ECs control the vascular tone and permeability, vascular SMC proliferation, leukocyte trafficking and adherence, inflammation, and thrombosis. Emerging evidence indicate that flow-sensitive miRNAs, known as mechano-miRNAs, regulate endothelial gene expression, being important players in angiogenesis, EC proliferation and function, and in endothelial dysfunction [56, 57••]. Several miRNAs, such as miR-126, miR-10a, miR-19a, miR-23b, miR-21, miR-663, miR-92a (and possibly other members of the miR-17-92 cluster), miR-143/145, miR-101, miR-712, miR-205, miR-155 (reviewed by Kumar et al. 2014), 146a, and miR-181b have been identified as mechano-miRNAs. These mechano-miRNAs mainly target key signaling pathways involved in EC cycle, inflammation, apoptosis, and NO signaling [57••]. Between all these mechano-miRNAs, some have antiatherogenic effects while others have proatherogenic effects. The antiatherogenic mechano-miRs are: miR-10a, miR-19a, miR-23b, miR-101, and miR-143/145. These miRNAs have been either increased by stable flow/laminar shear stress or decreased by disturbed flow/oscillatory shear stress in ECs. On the other hand, the proatherogenic mechano-miRs are as follows: miR-17-92 cluster, miR-92a, miR-663, miR-712, and miR-205. The latter miRNAs have been either increased by disturbed flow/oscillatory shear stress or decreased by stable flow/laminar shear stress in ECs and seem to induce endothelial dysfunction and proatherogenic responses [57••].

High shear stress-inducible miRNAs, such as miR-10a, miR-19a, and miR-23b, and laminar shear stress upregulated miR-146a and miR-181b may have vasoprotective and anti-inflammatory effects [58–60]. The anti-inflammatory effects of miR-146a and miR-181b are mediated through NF-κB pathway inhibition [61, 62]. Confirming the favorable effect of miR-146a and miR-181b in ameliorating endothelial inflammation, recent data have evidenced that delivery of miR-146a/181b packaged in E-selectin-targeting multistage vector (ESTA-MSV) MPs to ECs has caused downregulation of the expression of several chemokines, including CCL2, CCL5, CCL8, and CXCL9, and decreased adhesion of monocyte to ECs [63]. Thus, key molecules implicated in vascular tone regulation are targeted by several specific miRNAs. In addition, there are several mechano-miRs with dual role in atherosclerosis, such as miR-21, miR-126, and miR-155. Some of these mediate proatherogenic responses while others mediate antiatherogenic responses. This means that a single miRNA can target numerous mRNAs involved in both anti- and proatherogenic responses [64].

In addition to their role in vascular homeostasis, it is also known that ECs release mediators which modulate the function of the vascular SMCs in a manner that decisively influences vascular remodeling. It has been shown that endothelial-derived miR-143/145 can be transferred to SMCs via extracellular vesicles or MPs, and this vesicle-mediated transfer has prevented SMC de-differentiation [64]. Interestingly, SMCs can also transfer miR-143 and miR-145 to ECs, reducing the proliferation of ECs and thereby modulating angiogenesis [65]. Moreover, ECs can deliver miRNAs to monocytes. A recent study has reported that several anti-inflammatory microRNAs have been found elevated in endothelial-derived-extracellular vesicles-treated monocytes. In particular, miR-10a transferred to monocytic cells could repress the inflammatory signaling through targeting of several components of the NF-κB pathway [66].

MicroRNA in Hypertension-Associated Endothelial Dysfunction

The most important risk factor for the premature CVD is hypertension with a dysfunctional endothelium caused by the activation of ECs as a result of their constantly exposure to high intraluminal blood pressure. Thus, increased blood pressure alters EC phenotype and function [67]. Dysfunctional ECs participate in the pathogenesis of hypertension by several ways, including impaired vasodilatation, reduced bioavailability of NO, release of inflammatory and procoagulant mediators, and defective angiogenesis causing capillary rarefaction. A growing body of evidence indicates dysregulation of miRNAs to be a causative factor in endothelial dysfunction by affecting eNOs, endothelial repair, vessel wall angiogenesis, or expression of inflammatory molecules [56, 57••]. Also, the augmented oxidative stress generates the vascular dysfunction by the reduction of NO levels which impair the blood vessel vasodilation, another potential mechanism mediating the adverse effects of hypertension [68]. Thus, key molecules implicated in the vascular tone regulation have been shown to be targeted by several miRNAs.

In the last few years, many studies reported that changes in several plasmatic miRNA levels, as well as dysregulation of miRNA expression in tissue, are directly linked to CVD. Recently, N. Simionescu et al. have investigated miRNAs in sera and HDL obtained from the patients with/without hyperglycemia suffering from acute coronary syndrome or stable

angina [69]. Their results have revealed that miR-223, miR-92a, miR-486, miR-122, miR-125a, and miR-146a levels have been higher in the sera from hyperglycemic patients with acute coronary syndrome compared to sera from normoglycemic patients suffering from acute coronary syndrome. Moreover, miR-223 and miR-486 have been abounded in HDL2, and miR-92a in HDL3. Interestingly, these latter miRNA levels have been increased in HDL from hyperglycemic patients with acute coronary syndrome versus normoglycemic ones, the results discriminating between patients with acute coronary syndrome and patients with stable angina [69]. In addition, miR-486 and miR-92a have been associated with stable or vulnerable coronary artery disease [70]. The investigators have found higher levels of miR-486, miR-92a, and miR-122 in sera from patients with coronary artery disease [70]. Other five miRNAs, miR-125a-5p, miR-146a, miR-10a, miR-21, and miR-33a, have been detected in α-lipoprotein fraction from sera, and miR-33a has been found in β-lipoprotein fraction as well [71]. It is important to note that the circulating levels of miR-125a-5p and miR-146a have been increased in sera from hyperlipidemic and/or hyperglycemic patients [71]. Also, circulating levels of cardiac miRNAs, including miR-1, miR-133a, miR-208a, miR-208b, and miR-499, have been frequently reported as elevated in both coronary heart disease and heart failure, and have been proposed as candidate biomarkers that reflect the severity of myocardial injury [72].

In a prospective study aimed to investigate the association between circulating miRNAs and incident myocardial infarction, 3 miRNAs out of 19 miRNAs that have been quantified in the plasma of 820 participants were significantly related to incident myocardial infarction: miR-126, the master regulator of endothelial homeostasis and vascular integrity, showed a positive association, while miR-223 and miR-197 were inversely associated with disease risk [73]. Interestingly, miR-223 is antiangiogenic, preventing EC proliferation, at least in part via targeting β1 integrin [74]. Of note, circulating miR-223 has been identified as a biomarker and therapeutic target in inflammation, cancer, or obesity [75, 76]. In a small cohort of patients following myocardial infarction, the temporal profile of circulating levels of miR-1, miR-21, miR-29a, miR-133a, and miR-208 has been characterized and associated with the left ventricular remodeling [77].

In hypertensive rats showing signs of heart failure, the circulating levels of miR-16, miR-20b, miR-93, miR-106b, miR-223, and miR-423-5p were significantly increased. This effect was blunted after the treatment with anti-miR-208a, suggesting that circulating levels of miRNAs are responsive to therapeutic interventions and that these plasma miRNAs may serve as biomarkers of therapeutic efficacy and disease progression in hypertension-induced heart disease [78].

Therefore, the cardiovascular system is considered to be extremely sensitive to changes in miRNA levels. Accordingly, miRNAs might be important players in the pathogenesis of hypertension-associated endothelial dysfunction by the modulation of RAAS components, endothelial NO release, ROS production as well as by the regulation of inflammatory and angiogenic responses of ECs (Fig. 2).

MicroRNAs and Renin-Angiotensin-Aldosterone-System

RAAS plays a critical role in controlling arterial blood pressure, fluid and electrolyte balance, and vascular tone.

Excessive activation of RAAS is intricately involved in the etiology of hypertension [79], and pharmacological blockade of RAAS is a primary approach for the treatment of hypertension and of other vascular disorders [12]. Mounting evidences indicate that miRNAs interact with RAAS components, and these RAAS-related miRNAs take part in physiological and pathophysiological processes, acting either as mediators or targets of RAAS inhibitors [80–82].

The study by Marques et al. showed that miR-181a-5p binds the 3′UTR of renin mRNA, reducing its gene expression, and higher expression of renin mRNA in hypertensive individuals is accompanied by lower renal expression of miR-181a [83]. Also, the renin expression has been found to be controlled by miR-663 [83]. An enhanced sympathetically-induced renin synthesis has been mediated by diminution of miR-181a in a genetically hypertensive strain of mice [84].

ACE has been identified as a target of miR-145 from the miR-143/145 cluster—a major regulator of the contractile phenotype of vascular SMCs; pharmacological inhibition of either ACE or the AT_1R partially reversed the vascular dysfunction in miR-143/145-deficient mice [85]. Endothelial ACE has been found to be down-regulated by AMP-activated protein kinase by means of p53 phosphorylation and miR-143/145 upregulation [86••]. Moreover, stretch-activated ERK1/2 and up-regulated ACE contributed to miR-145 expression suppression in the vascular vessels exposed to elevated stretch, as occuring in hypertension [87]. These results suggest that the overexpression of miR-145 may inhibit the pathological vascular remodeling.

Ang II has previously been reported to regulate the expression of miR-29b, miR-129-3p, and miR-212 through mechanisms depending on Gαq/11 and ERK1/2 activation [88]. Likewise, recent data suggested that miR-132 and miR-212 are involved in Ang II-induced hypertension [89]. It is well known that Ang II regulates multiple aspects of EC function. Among many other stimuli, Ang II induces the expression of ET-1, a key endothelial transcription factor known to be involved in the regulation of endothelial inflammation, angiogenesis, and vascular remodeling [90]. A study by Zhu et al. revealed an important role of miR-155 in regulating Ang II and ET-1 signaling [91]. They proved that human umbilical vein endothelial cells (HUVECs) highly express miR-155 and miR-221, and they provided evidence that miR-155 targets

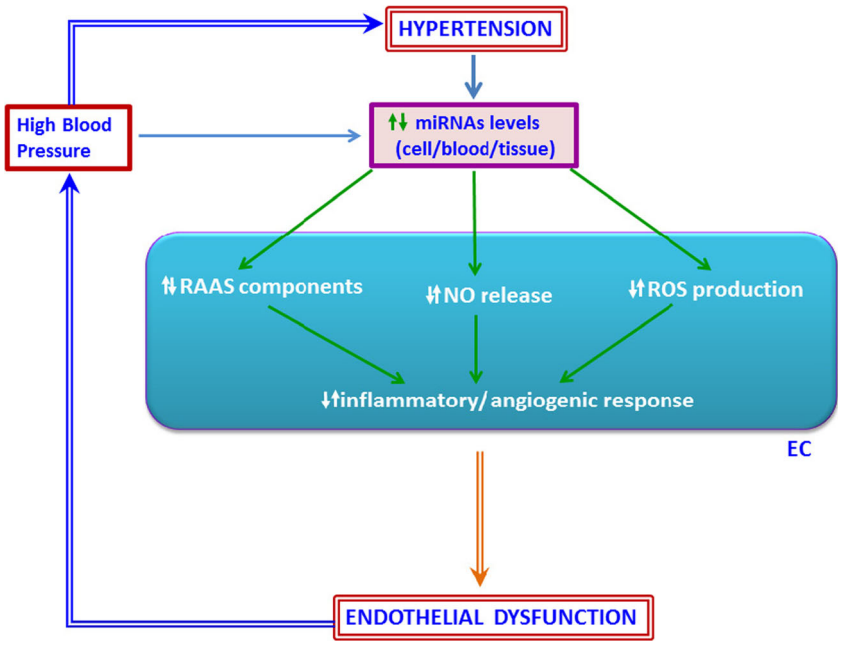

Fig. 2 The role of miRNAs in endothelial cell dysfunction in relation to hypertension

AT_1R, and miR-155 and miR-221/222 cluster are able to target ET-1. Moreover, ET-1 and its downstream genes, including VCAM1, MCP1, and Fms-like tyrosine kinase 1 (FLT-1), have been up-regulated in Ang II-stimulated HUVECs, and this effect has been partially reversed by overexpression of miR-155 and miR-221/222; also, overexpression of miR-155 diminished Ang II-induced AT_1R-dependent endothelial migration [73]. Other researchers showed that miR-155-5p inhibited AT_1R expression in HUVECs, and miR-155 reduced Ang II-induced ERK1/2 activation [92]. Also, miR-155 expression in HUVECs attenuated Ang II-induced apoptotic factors by targeting the AT_1R [75]. Recent studies evidenced that the overexpression of miR-155 has been able to inhibit mRNA and AT_1R protein expression in Ang II-treated hypertrophic cardiomyocyte [93]. Given that AT_1R signaling is upstream of cardiac and vascular hypertrophy, it is likely that therapeutic targeting of miR-155 to improve hypertensive condition by modulating/downregulating AT_1R expression. Although the above findings suggest a protective role for miR-155 in EC function, other researchers reported that miR-155 targeted the 3′ UTR of eNOS in HUVECs, suggesting that this may promote EC dysfunction. Thus, miR-155 overexpression decreased, whereas miR-155 inhibition increased eNOS expression and NO production in ECs and acetylcholine-induced endothelium-dependent vasorelaxation in human internal mammary arteries [94].

Of note, miRNAs have been found to modulate ACE2 signaling with favorable effects for cardiovascular system, suggesting that targeting these miRNAs could lead to novel therapeutic strategies to prevent or treat hypertension and its related vascular diseases [95•]. Kemp et al. showed that miR-483-3p is involved in the regulation of AT_2R, angiotensinogen (AGT), ACE-1, and ACE-2, all the key components of RAAS [96•]. They established that miR-483-3p overexpression down-regulated the expression of AGT, ACE-1, and ACE-2 proteins known as modulators of pathophysiology of vascular endothelium and SMC. Several single nucleotide polymorphisms (SNPs) within the miRNA binding sites of RAAS genes have been found associated with hypertension. For instance, A1166C polymorphism in the 3′-UTR of the human AT_1R (SNP ID: rs5186) overlaps with the miR-155 target site in this gene. For the 1166C allele, the base pairing complementarity of miR-155 with the AT_1R mRNA sequence is affected, and the ability of miR-155 to suppress AT_1R expression is diminished. Thus, AT_1R-1166CC carrier state may lead to enhanced AT_1R expression, elevated blood pressure, and increased risk for vascular pathologies [97, 98].

MicroRNAs Modulating Nitric Oxide Release

NO is known as important regulator of vasodilatation and blood flow and a real protector of cardiovascular system by improving of endothelial function. It has been attested by many in vivo and in vitro studies that NO release can be modulated by miRNAs, and the vascular complications in hypertension are often associated with the impairment of endothelial function-related miRNAs regulation. In hypertension, the alterations of flow conditions modulate the miRNA levels in ECs. As an example in this respect, one study showed that miR-122 contributes to the endothelial dysfunction in hypertension by diminishing L-arginine and NO metabolism [99]. It is well known that L-arginine is the precursor and essential component for NO synthesis, NO induces vasodilation, and Solute Carrier Family 7 Member 1 (SLC7A1) is a

transporter gene for L-arginine and normal NO metabolism [100]. It seems that the miR-122 binds the 3′UTR of SLC7A1, and polymorphism in this site (on the level of SLC7A1) generates decreased levels of SLC7A1 that subsequently causes lower levels of NO and the endothelial dysfunction in hypertensive patients [99]. Two other miRNAs have been proposed as biomarkers for the endothelial cell dysfunction in hypertension: miR-182 as a novel target of the crosstalk between ECs and cardiomyocytes [101] and miR-155 as a new therapeutic approach in the endothelial dysfunction improvement during CVD progression. Specifically, it has been shown that the increase of miR-182 expression induces Akt/mTORC pathway activation and subsequently the endothelial dysfunction by decreasing NO release, and the increase of miR-155 expression is inversely related to eNOS expression and NO release. Concerning miR-155, since this can modulate the expression of two signaling molecules essential for the vascular homeostasis, namely eNOS and AT_1R [94, 102], it is likely that this miRNA to take part in the pathogenesis of hypertension. Sun et al. reported that miR-155 is an essential modulator of eNOS expression and crucial factor in the endothelium-dependent vasorelaxation as well [94]. As described, miR-155 binds to the 3′ end of its target mRNA to control the eNOS expression, thus contributing to bioavailability of NO responsible for maintaining homeostasis. Accordingly, miR-155 can be seen as pro-inflammatory factor, since NO has anti-inflammatory properties, inhibiting leukocyte adhesion and preventing in this way the vascular inflammation and impaired vasodilation in hypertension. Another mediator of NO release regulation is miR-221/222 cluster that appears to be responsible for reduced NO secretion and lower NOS3 mRNA expression in ECs [103]. The miR-221/222 cluster is also involved in maintaining endothelial integrity and supporting quiescent EC phenotype. Several studies evidenced the negative regulatory effects of miRNA-221/222 on several key genes, such as cyclin-dependent kinase cell cycle regulators $p21^{Cip1}$ and $p27^{Kip1}$, transcription factors Ets1 and Ets2, signal transducer, and activator STAT5a [104]. Of note, the miR-221/222 cluster suppresses endothelial production of matrix metalloproteinases (MMPs), several key adhesion modulators (such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), integrin-β3), and eNOS, promoting endothelial dysfunction. Also, up-regulated vascular expression of miR-221/222, in early atherogenic stages, suppresses the angiogenic recruitment of ECs, increasing EC apoptosis and endothelial dysfunction [105]. Additionally, in HUVECs exposed to atorvastatin, simvastatin, or ezetimibe, atorvastatin decreased the miR-221 and miR-222 expressions, while simvastatin and ezetimibe reduced only miR-221 expression. Since statins led to downregulation of miR-221/222, these have been considered as being responsible for increasing NOS3 mRNA levels [106]. These results bring new data concerning the contribution of regulatory miR-221/222 on NO release induction mediated by statins. Also, authors conclude that even though ezetimibe has not modulated NO levels and NOS3mRNA expression, the downregulation of miR-221 could involve potential effects on endothelial function. Moreover, no influence has been observed on the miR-1303 expression after treatments with these three cholesterol-lowering drugs: atorvastatin and simvastatin (inhibitors of cholesterol synthesis) and ezetimibe (inhibitor of cholesterol absorption) [106].

As another recent study has reported, the eNOS expression may also be regulated by miR-24. As it has been shown, the core promoter region of the human eNOS gene, located in 7q35-36, contains multiple *cis*-acting elements, including high-affinity binding sites for specificity the protein 1 (Sp1) transcription factor, and miR-24 targets Sp1 inhibiting eNOS expression and induces EC proliferation [107].

Other miRNAs, such as miR-9 and miR-126, have been associated with the endothelial dysfunction in hypertensive patients. These presented different expressions when were evaluated comparative to healthy subjects [108•]. On the other hand, the miR-143/145 cluster has been found to have an important role in the pathobiology of pulmonary arterial hypertension, being implicated in the regulation of both cellular and exosome-dependent responses in ECs in pulmonary arteries. In this regard, it has been exhibited that the inhibition of miR-143/145 prevented the experimental pulmonary hypertension [109]. Other data suggested that miR-30a accelerates arteriolar branching by downregulation of endothelial delta-like 4 (Dll4) expression, controlling in this way the EC behavior in hypertension. Thus, the prevention of microvascular rarefaction, a hallmark of fixed essential hypertension, by the activation of angiogenic processes with miR-30a could lower the blood pressure [110]. It has recently been shown that miR-26a has an important role in the EC apoptosis and also it is dysregulated in atherosclerosis and directly targets the transient receptor potential protein homologue TRPC6 in vascular ECs [107]. Another study has established that miR-27b is a key player in the endothelial function and NO release. It has also been proposed a potential mechanism for the control of Hsp90-eNOS and NO signaling during modulation of peroxisome proliferator-activated receptor gamma (PPARγ) by miR-27b. These findings may provide new insights for the treatment of pulmonary arterial hypertension [111]. Moreover, these altered miRNAs may be new clinical prognostic indices of target-organ damage in hypertensive patients.

MicroRNAs Controlling Oxidative Stress

Oxidative stress plays a crucial role in CVD, such as hypertension, heart failure, cardiac hypertrophy, and atherosclerosis. In the vascular remodeling in hypertension, the oxidative stress has been associated with several aspects like the

endothelial dysfunction, inflammation, cell migration, apoptosis, and angiogenesis [112, 113]. Elevated levels of ROS damage both transcriptional and post-translational activity of vascular ECs and also induce changes in mitochondrial DNA and biogenesis. Subsequently, all these effects deteriorate global antioxidant defense processes.

In miRNA biogenesis, Dicer cleaves miRNA precursors generating short double-stranded miRNA duplexes. The inactive strand or carrier strand called miRNA* (miRNA star) or passenger strand that is usually degraded seems to be selectively stabilized by oxidative stress. In macrophages, the NF-κB pathway is modulated by several oxidative stress-responsive miRNAs: miR-27a, miR-27b, miR-29b, miR-24, and miR-21, affecting cell functions [114]. Also, there are many miRNAs found to be key players in regulating oxidative stress responses in numerous vascular diseases. Nevertheless, for the time being there are few reports about miRNA expression signatures in oxidative stress-associated endothelial dysfunction in hypertension. It has recently been demonstrated that miR-1 targets redox-related proteins: Cu/Zn superoxide dismutase (SOD1), glutamate-cysteine ligase (Gclc), and Glucose-6-phosphate dehydrogenase (G6PD), and it post-transcriptionally represses the expressions of these antioxidant genes having potential complementary sites in the 3′ UTRs of these genes [115••]. This process contributes to increasing of ROS levels and intensifies the vulnerability to oxidative stress of the heart in miR-1 transgenic mice [115••]. As a result, this report demonstrates that enhanced miR-1 levels create the alterations in the expression of proteins related to oxidative stress, which could contribute to heart dysfunction and hence possible to endothelial vascular dysfunction. In addition, it has been attested that rats with experimental infarction displayed elevated miR-1 levels. Consistent with this idea, another study has demonstrated that mice have significantly reduced cardiac function when miR-1 is overexpressed. In patients suffering from coronary artery disease associated with oxidative stress, the miR-1 levels have also been discovered increased. In addition, the antioxidant treatment controls oxidant/antioxidant level, which may directly regulate several miRNA levels, especially miR-1. The miR-1 along with other miRNAs such as miR-499, miR-133a, and miR-133b seems to unbalance the ratio of oxidant to antioxidant defense in the myocardium during diabetic cardiomyopathy [116]. In agreement with these findings, miR-200c, miR-200a, and miR-141 have been found to be major effectors of oxidative stress-induced biological responses in ECs. These have been very well expressed in HUVECs exposed to H_2O_2 for different periods of time [117]. The potential action mechanism of miR-200c, miR-200a, and miR-141 is targeting p38α MAPK, a signaling molecule that contributes to regulation of cellular responses to stress [118] as well as to the control of proliferation and survival of many cell types [119].

Of note, the oxidative stress regulates a panel of miRNAs that target the 3′UTR of silent mating type information regulation 2 homologue (sirtuin 1 or SIRT1) mRNA. There are miRNAs that target SIRT1 and miRNAs that indirectly regulate it. The miRNAs able of targeting SIRT1 seem to be involved in the atherosclerosis development. For example, miR-217 has been found to be involved in the vascular diseases, mainly in atherosclerosis and abdominal aortic aneurysm, and it has been negatively correlated with SIRT1 expression in human atherosclerotic plaques, Forkhead box protein O1 (FoxO1) acetylation status, and with eNOS expression as well [120]. Many other studies showed a positive correlation between the circulating miR-21 levels and blood pressure in hypertensive patients. Anyway, a very recent study has observed a significantly reduction of blood pressure and cardiac hypertrophy after the recombinant adeno-associated virus-mediated delivery of miR-21 in SHR [121]. This report discloses a positive function of miR-21 in the mitochondrial translation, which is sufficient to decrease blood pressure and improve cardiac hypertrophy in SHR. An explanation that comes from these authors states that the induction of miR-21 regulates the ROS generation in mitochondria this being a part of a compensatory program [121]. Again these data lead us to think that the discovering of new miRNA-based therapies could help in the future the hypertensive patients.

MicroRNA Function in Vascular Inflammation

Since the inflammation contributes to different vascular diseases, it is critical to know and understand the action mechanism of inflammatory markers and the ways by which they are regulated by other factors as well. Inflammation is initiated by oxidative stress through the activation of transcription factors like NF-κB. In in vivo hypertension, modified levels of CRP have been correlated with oxidative stress intensity within inflammatory cells [122]. Inflammation generates the endothelial dysfunction therefore promoting hypertension. Every inflammatory process persists until the pathogens are eliminated and the tissue is completely repaired. In addition, the persistent inflammation can cause overproduction of ROS. In some chronic inflammatory diseases, inflammatory stimuli induce the abnormal expression of miRNAs. On the other hand, in 2008, Harris et al. stated the involvement of miRNAs in EC activation and dysfunction. They demonstrated that miR-126 decreases VCAM-1 expression in ECs by binding to its 3′ UTR, and therefore the leukocyte–EC interactions in response to TNF-α have been reduced [123]. The miR-126 expression is mediated, in part, by the E26 transformation-specific sequence (Ets) factors, Ets-1 and Ets-2, with role in the regulation of EC differentiation and vascular

inflammation [124••]. The levels of circulating miR-126 have been assayed to be lower in young patients with stroke and patients with coronary artery disease [125]. Additionally, other several specific miRNAs have been reported to regulate the EC function and vascular inflammation as response to a variety of pathophysiological stimuli: miR-17-3p, miR-31, miR-181b, miR-146, miR-10a, and miR-92a. As for miR-17-3p, it targets the 3′ UTRs of ICAM-1, while miR-31 targets E-selectin. They regulate EC activation induced by cytokine TNF-α, an effect that subsequently inhibits the leukocyte adhesion to activated ECs [57••]. Also, both in vitro and in vivo studies reported that the overexpression of miR-181b inhibits importin-α3 expression and enhances the set of NF-κB-responsive genes such as VCAM-1 and E-selectin in ECs [62]. More recently, Cheng et al. revealed that pro-inflammatory cytokines have reduced the expressions of miR-146a and miR-146b in ECs [126]. As a consequence, the overexpressions of miR-146a and miR-146b have ameliorated the endothelial activation by the inhibition of NF-κB and MAPK pathways after directly targeting of the 3′ UTR of TNF-receptor-associated factor 6 (TRAF6) and HuR. TRAF6 and HuR are RNA-binding proteins which exert inhibitory effects on NF-κB activation and eNOS expression, respectively. Thus, miR-146 is considered another critical component of a negative-feedback loop that controls the endothelial activation and dysfunction. Besides, several data obtained in patients with chronic obstructive pulmonary disease have revealed that the inflammatory cytokines increased miR-146a levels that target COX2 gene [127] while, in CD4+ T- and CD8+ T cells from patients with severe asthma, the expression of miR-146a has been significantly reduced [128]. Another important study uncovered that the shear stress regulates the expressions of miR-10a and miR-92a [129]. The miR-10a reduces NF-κB activation by targeting the 3′ UTR of MAPK-7 and the β-transducin-repeat-containing gene (β TRC), while miR-92a is involved in the regulation of endothelial activation by targeting the transcription factors KLF2 and KLF4 9. Consistent with these data, an inverse correlation has been found between miR-10a, miR-92a expressions and NF-κB, KLF2, KLF4 expressions in athero sites from swine aortic arch relative to the protected thoracic aorta. Another in vivo evidence has discovered that miR-21 has a positive effect in hypertension and atherosclerosis process. Thus, in their study, Zhang et al. showed that the diastolic blood pressure has been increased and acetylcholine-induced endothelium-dependent relaxation of the aorta has been reduced in miR-21 endothelial-specific knockout mice [130]. Also, the deficiency in miR-21 diminished elastin content and enhanced the intima-media wall thickness of the thoracic aorta. As for the miR-21 action molecular mechanism, it has been established that the deletion of miR-21 increases the expressions of Smad7, connective tissue growth factor (CTGF), MMP-2, and MMP-10, and decreases the expressions of Smad2, Smad5, and tissue inhibitor of MMP-4. The data from this interesting study evoked that endothelial miR-21 may play a decisive role in the vascular remodeling through regulating transforming growth factor-β_1 (TGF-β_1) signaling [130]. Furthermore, miR-125a-5p and miR-125b-5p have also been shown to decrease in SHR, as a consequence of increasing of ET-1 expression known as a potent vasoconstrictive peptide that promotes the endothelial inflammation and atherosclerosis [131]. Also, miR-155 and miR-221/222 directly target Ets-1, an effect that reduces VCAM-1, MCP-1, and Fms-like tyrosine kinase 1 (FLT-1) expressions with a concordant reduction in leukocyte–EC interactions and EC migration [91]. It has recently been made known that the miR-712/205 family, which is up-regulated by disturbed flow, increases the vascular permeability and contributes to endothelial inflammation by targeting tissue inhibitor of MMP-3 [57••].

In addition to all of these data, another study exhibited that elevated levels of IL-6 led to reduced bone morphogenetic protein receptor type II (BMPR2) expression in idiopathic pulmonary arterial hypertension through the activation of miR-17/92 cluster [132]. In agreement with these data, another report has analyzed the miR-663 expression in HUVECs exposed to proatherogenic oxidized phospholipids. As a result, the increase in mir-663 expression has been correlated to stimulation of VEGF and activation of activating transcription factor 4 (ATF4) [133]. Generally, it is largely known that inflammatory cytokines such as IL-17, IL-6, and VEGF contribute to hypertension, likely by both altering blood pressure and tissue damage. Recently, in vivo studies showed that miR-637 has anti-inflammatory effect by lowering CRP circulating levels. Since miRNAs have a key role in posttranscriptional gene regulation mainly by acting as repressors, several targets for mir-637 such as osterix and collagen type IV alpha 1 (COL4A1) have been reported [134, 135]. It is possible that miR-637 to target other inflammatory pathways in order to decrease the CRP levels in other pathological situations such as hypertension.

Finally, all these data suggest that miRNAs may be important regulators of vascular function in human disease, particularly in hypertension, and in the same time they may be real targets for therapies. It would be fascinating to disclose whether in vivo administration of any of these miRNAs modulate the pathogenesis of vascular diseases.

MicroRNA Function in Vascular Angiogenesis

The angiogenic response is often regulated by miRNAs, but their expression may also be controlled by pro-angiogenic factors, such as VEGF and basic fibroblast growth factors (bFGF). In ECs, Dicer, the terminal endonuclease responsible for the generation of miRNAs, has a major role in angiogenesis. In both in vitro and in vivo, lacking of this enzyme results in an extremely dysregulated angiogenesis. It has been shown

that the diminution of endothelial miRNAs by Dicer inactivation decreased postnatal angiogenic response to different stimuli, including exogenous VEGF, wound healing, limb ischemia, and tumors. In homeostasis and disease, as well as in many growth aspects, the posttranscriptional gene regulation by miRNAs is fundamental. It has been confirmed by several times that miRNAs regulate the new blood vessel formation in maladies like cancer [136] and several hypertension-related complications [47]. Interestingly, the postnatal angiogenesis has been regulated by endothelial miRNAs, and VEGF induced the expression of miRNAs implicated in the angiogenic response control [103]. The several specific miRNAs involved in angiogenesis have been recently identified. In vivo studies attested that miR-329 inhibits the CD146 expression in blood vessels and attenuates neovascularisation [47]. Also, the hypoxia-dependent upregulation of miR-24 led to moderated capillary density through targeting GATA2 (an endothelium-enriched transcription factor) and PAK4 (a p21-activated kinase) and decreasing their expression in ECs [137]. Overexpression of miR-217 in young ECs has induced deficient angiogenesis, and the inhibition of miR-217 in old ECs led to augmented angiogenesis [138]. In young HUVECs, human aortic ECs, and human coronary artery ECs, miR-217 has promoted a premature senescence-like phenotype and conducted to the impairment of angiogenesis via inhibition of SIRT1 and modulation of FoxO1 and eNOS acetylation. The decline of angiogenesis inside microvessels named rarefaction generates the injury of target organs, a major pathogenic feature and complication of hypertension. Recently, another in vivo study has demonstrated that in the right ventricle, the miR-126 upregulation improves the microvessel density in the pulmonary arterial hypertension [139]. It has been shown that the miR-126 downregulation increased sprouty-related, EVH1 domain-containing protein 1 (Spread-1) and phosphoinositol-3 kinase regulatory subunit 2 (PI3KR2), conducting to RAF and MAPK inactivation and VEGF pathway inhibition. In addition, it has been found that miR-182 upregulation plays an important role in the hypertrophic response induced by angiogenesis through downregulation of branched chain amino acid transaminase 2 (Bcat2), adenylate cyclase 6 (Adcy6), and FoxO3 [101]. Besides, the exercise training promotes the peripheral revascularization in hypertension, associated with the regulation of several miRNAs. Thus, the clarification of connection between the exercise training and miRNAs in the pathogenesis of hypertension is required to understand how exercise modulates the cardiovascular system at genetic level [140]. Related to the capillary rarefaction that is known to amplify vascular dysfunction in hypertension, two of most frequent miRNAs, miR-16 and miR-21, have been found to be increased, while their targets VEGF and Bcl-2 have been decreased. Furthermore, it has been established by experimental results that the exercise training lowers the miR-16 and miR-21 expression and increases miR-126 expression, associated with the revascularization in hypertension [141]. Moreover, it has been shown that aerobic training induces an increase of mir-126 expression, related to exercise-induced cardiac angiogenesis, by indirect regulation of the VEGF pathway and direct regulation of its targets that converged in an activation of angiogenic pathways, such as MAPK and PI3K/Akt/eNOS [142]. The levels of miR-126 and miR-9 both analyzed in hypertensive patients have been found to be significantly diminished [108•]. Moreover, the echocardiography measurements showed that left ventricle mass index can be positively correlated with miR-9. These findings have been comparable with those obtained in animal studies stating that miR-9 is a negative regulator of cardiac hypertrophy [143]. The miR-9 suppresses myocardin expression which is a downstream mediator of nuclear factor of activated T cells c3 (NFATc3) in the hypertrophic cascades. In addition, it has been discovered that the levels of miR-126 and miR-9 can be positively related to the 24-h mean pulse pressure, which can predict advanced target organ damage in hypertension [144]. Another study detected the increases of miR-505 expression in the hypertensive subjects and animals [145]. The direct target of miR-505 has been established to be the fibroblast growth factor 18 (FGF18), a pro-angiogenic factor responsible for the antiagiogenic effects of miR-505.

These exciting studies suggest that the adjustment of miRNA expression and function by different approaches may lead to the regulation of angiogenic response in hypertension. Thus, a novel method for the treatment of disorders associated with abnormal pathological angiogenesis, such as hypertension, might be based on the antagonism of key miRNAs.

All these miRNAs mentioned here with essential role in endothelial dysfunction in hypertension by direct targeting of multiple components of RASS, NO release, ROS production, and also of inflammatory and angiogenic responses in ECs are summarized and displayed in the Table 1.

Above all these data, it is important to mention that miRNAs offer many features for making them effective pharmacological targets in hypertension-associated endothelial dysfunction. Anyway, growing evidence in the field of miRNAs could be added to this current understanding so that miRNAs can be used as diagnostic biomarkers and therapeutic targets for hypertensive patients.

MicroRNAs as Mediators of Intercellular Communication

There are important studies which demonstrate that miRNAs exist in both cellular and extracellular space, including plasma/serum, saliva, and urine. Some circulating miRNAs in blood have been successfully revealed as biomarkers for several cancers, CVD, brain injury, and liver injury [146]. miRNAs detected in blood, known as "circulating

Table 1 RASS components, NO release, ROS production, inflammation, angiogenesis-related microRNAs in hypertension

miRNAs	Affected signaling pathways/targets	miRNA functions in endothelial dysfunction and hypertension	References
MicroRNAs and renin-angiotensin-aldosterone-system			
↓ miR-181a-5p	↑ renin	Increases blood pressure in hypertensive patients and mice	[83, 84]
↓ miR-663	↑ renin	Increases blood pressure in hypertensive patients	[83]
↓ miR-143/145	↑ AT1R, ACE	Induces vascular dysfunction in miR-143/145-deficient mice	[85, 86••]
↓ miR-145	↑ ACE, ERK1/2	Induces vascular dysfunction in vessels exposed to elevated stretch	[86••]
↑ miR-29b ↑ miR-129-3p ↑ miR-212	↑ Gαq/11, ERK1/2 activation	Increase blood pressure and endothelial inflammation	[88]
↓ miR-155-5p	↑ AT1R (AT_1R-1166CC carrier state), ERK1/2 ↑ ET-1, VCAM1 ↑ MCP1, FLT-1	Induces vascular hypertrophy Modulates endothelial migration in HUVECs	[93, 113] [91]
↓ miR-221/222	↑ ET-1, VCAM1 ↑ MCP1, FLT-1	Modulates endothelial migration in HUVECs	[91]
↓ miR-483-3p	↑ AT_2R, AGT, ↑ ACE-1 ACE-2,	Modulates RAS component levels	[96•]
MicroRNAs modulating nitric oxide release			
↑ miR-155	↓ eNOS	Decreases NO release in HUVECs and impairs endothelium--dependent vasodilation Induces leukocyte adhesion and vascular inflammation	[94]
↑ miR-122	↓ SLC7A1 ↓ L-arginine	Reduces NO levels and induces endothelial dysfunction in hypertensive patients	[99, 100]
↑ miR-182	↓ Akt/mTORC	Targets the crosstalk between ECs and cardiomyocytes Induces endothelial dysfunction by decreasing NO release	[101]
↑ miR-24	↓ Sp1, eNOS	Decreases NO release and induces EC proliferation	[107]
↑ miR-221/222	↓ eNOS ↓ $p21^{Cip1}$, $p27^{Kip1}$ ↓ Ets1, Ets2, STAT5a	Lowers NO secretion and NOS3 expression in ECs	[103, 104, 106]
↑ miR-27b	↓ Hsp90-eNOS ↓ PPARγ	Diminishes NO generation and causes pulmonary arterial hypertension	[111]
↑ miR-217	↓ eNOS	Decreases NO release in ECs from abdominal aorta	[120]
↓ miR-146a ↓ miR-146b	↑ TRAF6, HuR ↓ eNOS	Stimulate EC activation and dysfunction	[126]
MicroRNAs controlling oxidative stress			
↑ miR-27a ↑ mirR-27b ↑ miR-29b ↑ miR-24	↑ NF-κB pathway	Increase ROS production and induce endothelial dysfunction	[114]
↑ miR-21	↑ NF-κB pathway	Increases ROS production and induces endothelial dysfunction Increases blood pressure in hypertensive patients and rats	[114] [121]
↑ miR-1	↑ Cu/Zn , SOD1 ↑ Gclc, G6PD	Generates increase of ROS levels and induces endothelial dysfunction	[115••]
miR-499, miR-133a, miR-133b	?	Unbalance the ratio of oxidant to antioxidant defense	[116]
↑ miR-200c ↑ miR-200a ↑ miR-141	↑ p38α MAPK	Increase ROS levels in HUVECs Effectors of oxidative stress-induced biological responses in ECs	[117–119]
↑ miR-217	↓ SIRT1, FoxO1	Increases ROS production and contributes to abdominal aortic aneurysm	[120]
MicroRNA function in vascular inflammation			

Table 1 (continued)

miRNAs	Affected signaling pathways/targets	miRNA functions in endothelial dysfunction and hypertension	References
↓ miR-126	↑ VCAM-1	Increases leukocyte–EC interaction generating endothelial activation and inflammation	[123, 124••, 125]
↓ miR17-3p	↑ ICAM-1	Enhances leukocyte adhesion to activated ECs	[57••]
↓ miR-31	↑ E-selectin	Enhances leukocyte adhesion to activated ECs	[57••]
↑ miR-181b	↓ importin-α3 ↑ VCAM-1 ↑ E-selectin	Induces EC inflammation in vitro and in vivo models	[57••, 62]
↓ miR-146a	↑ TRAF6, HuR	Stimulate EC activation and dysfunction	[57••]
↓ miR-146b	↑ NF-κB , MAPK ↓ eNOS		[126]
↓ miR-10a	↑ MAPK-7, β TRC ↑ NF-κB ↑ KLF2, KLF4	Promotes EC activation in athero sites from swine aortic arch	[129]
↓ miR-92a	↑ KLF2, KLF4 9	Promotes EC activation in athero sites from swine aortic arch	[129]
↓ miR-21	↑ Smad7, CTGF ↑ MMP-2, MMP-10 ↓ Smad2, Smad5 ↓ tissue inhibitor of MMP-4 ↓ TGF-β_1	Increases diastolic blood pressure and impairs endothelium-dependent relaxation of the aorta Diminishes elastin content and enhances the intima-media wall thickness of the thoracic aorta Modulates vascular remodeling in hypertension and atherosclerosis process	[130]
↓ miR-125a-5p ↓ miR-125b-5p	↑ ET-1	Promote endothelial inflammation and atherosclerosis in SHR	[131]
↓ miR-155 ↓ miR-221/222	↑ Ets-1, VCAM-1 ↑ MCP-1, FLT-1	Increase leukocyte–EC interactions and EC migration	[91]
↑ miR-712/205	↓ tissue inhibitor of MMP-3	Increases vascular permeability and contributes to endothelial inflammation	[57••]
↑ miR-17/92	↓ BMPR2 ↑ IL-6	Provokes endothelial inflammation in idiopathic pulmonary arterial hypertension	[132]
↑ miR-663	↑ ATF4, VEGF	Produces activation in HUVECs	[133]
↓ miR-637	↑ Osterix, COL4A1, ↑ CRP	Activates inflammatory pathways by increasing CRP	[134, 135]
MicroRNA function in vascular angiogenesis			
↑ miR-329	↓ CD146	Attenuates neovascularisation	[47]
↑ miR-24	↓ GATA2, PAK4	Decreases capillary density and impairs angiogenesis	[137]
↑ miR-217	↓ SIRT1, FoxO1 ↓ eNOS acetylation	Promotes a premature senescence-like phenotype in ECs and conducts to an impairment in angiogenesis	[138]
↓ miR-126	↑ SPRED-1, PI3KR2 ↓ RAF, MAPK ↓ VEGF ↓ PI3K/Akt/eNOS	Impairs the microvessel density in the pulmonary arterial hypertension	[108•, 139] [142]
↑ miR-182	↓ Bcat2, Adcy6 ↓ FoxO3	Contributes to angiogenesis-induced hypertrophic response	[101]
↑ miR-16 ↑ miR-21	↓ VEGF, Bcl-2	Generate capillary rarefaction and amplify vascular dysfunction	[141]
↓ miR-9	↑ myocardin ↑ NFATc3	Modulates cardiac hypertrophy	[108•, 143, 144]
↑ miR-505	↓ FGF18	Reduces angiogenesis in hypertensive subjects and animals	[145]

miRNAs," can originate from dying cells, such as necrotic cardiomyocytes following myocardial infarction, or can be actively secreted from living cells, acting as paracrine factors.

To our knowledge, the mechanism of miRNA release from the origin cells into extracellular space has not yet been described. It is possible that some of the miRNAs that convey specific information to be exported or released from cells in response to biological stimuli.

Several studies attested that miRNAs carry out biological function outside the cell, mediating cell-cell communication.

Also, the origin of circulating miRNAs is not yet well understood. It is believed that most of miRNAs are derived from blood cells, and some of them even from tissue such as heart, lung, liver, and kidney. In order to protect themselves against circulating RNase degradation, miRNAs associate with the extracellular vesicles (exosomes, microvesicles (MVs), or microparticles (MPs) and apoptotic bodies) and protein (AGO2) or lipoprotein complexes (LDL, HDL) [147]. Circulating miRNAs packed in different carrier molecules are subsequent transferred into recipient cells modifying their function [148, 149]. Not all miRNAs are loaded into extracellular vesicles or lipoprotein complexes, some of them are kept within the cell. The key step of intercellular communication is the selective export of a precise miRNA from the cell [150]. The process of sorting and export of miRNAs is ATP-dependent and it responses to extracellular conditions [147]. The exosomal export of miRNAs could be dependent on the ceramide pathway and linked directly with intracellular miRNA levels as well [147]. In addition, packing of miRNA into specific extracellular transport carrier can be influenced by disease. It has been shown that the levels of miRNAs in platelet-derived microparticles (PMPs) isolated from blood of patients with chronic coronary heart disease or acute coronary syndrome and from healthy subjects are significantly different [151]. In some cases, precursors of mature miRNAs have also been released. In addition, many studies have reported that functional miRNAs are transferred by MVs into recipient cells [148, 152, 153], mediating cell-cell communication. The cell-cell communication is facilitated mainly by intravesicular and extravesicular miRNA transport and sometimes by connexin-dependent miRNA intercellular transfer via gap junction. It has recently been described that compared with MV-dependent miRNA intercellular transport (~0.7 %), the percentage of intercellular transport of miRNAs by gap junctions is much higher [154]. Many studies attested the role of miRNAs in both local and systemic communication, especially in the case of adipocytes [155, 156] and tumor cells [136, 149, 157]. Thus, specific circulating miRNA profiles have been uncovered in plasma from obese subjects, suggesting that some of these miRNAs may modulate the systemic metabolic response [158]. More importantly, concerning miRNA role in local communication, the most recent studies have revealed that adipocyte-derived MVs contain miRNAs that are transported into macrophages. Moreover, it has been proved that secreted miRNAs by this type of MVs may target neighboring cells and perform regulatory functions in them [159]. In particular, it has been demonstrated that MVs derived from rat adipocytes or plasma harboring the glycosylphosphatidylinositol (GPI)-anchored proteins, Gce1 and CD73, contain specific transcripts and miRNAs that are both transferred into and expressed in acceptor adipocytes and are involved in the upregulation of lipogenesis and cell size [155]. Also, many miRNAs play important roles in regulating inflammation in adipose tissue [160]. Accordingly, under lipopolysaccharide stimulation mimicking an inflammatory state, an altered expression pattern of miRNAs in both adipocytes and macrophages has been evidenced, by comparison with the basal conditions [156]. The authors conclude that knowing miRNA profiles associated with different pathways of adipocytes and macrophages activation may provide a new spectrum of biomarkers and therapeutic approaches to diminish macrophage infiltration in obese adipose tissue and the onset of obesity-associated inflammation. For example, variations regarding miR-221/222 and miR-155 may participate in the crosstalk between obesity-related inflammation, insulin resistance, and other obesity-associated morbidities [156]. Moreover, in adipose tissue samples from morbid obese subjects obtained before and after bariatric surgery-induced weight loss, analyses of those miRNAs exhibiting dramatic changes in vitro revealed a reduction of their expressions after surgery-induced weight loss, together with the subsequent metabolic improvement and decreased inflammation [156].

Conclusion

Knowing the essential role of the endothelium in the control of vascular function, inflammation, thrombosis, and proliferation, it is obvious that the endothelial cell dysfunction involves the alterations of these normal endothelial functions. In hypertension, the endothelial dysfunction there is on the level of both resistance and conduit arteries and it is the result of the amplification of NO degradation by the interaction between NO and superoxide anions. There are growing evidence concerning association between hypertension and endothelial dysfunction but with respect to the mechanisms of hypertension-associated endothelial dysfunction important pieces of the puzzle are still missing.

The idea that miRNAs are critical regulators of various gene expressions may be a new hope for understanding the relation hypertension-endothelial dysfunction. In particular, miRNAs are important regulators of vascular function and hypertension, thereby serving as diagnostic and therapeutic targets of the vascular disease. The knowledge of miRNA biology has not yet been perfected, and the identification of specific target genes of miRNAs in each cell type and organ continues to be a major challenge in understanding the role of miRNAs. The finding of interactions between specific miRNAs and RAAS components, NO release, ROS production, and identification of their role in the inflammatory and angiogenic responses, and further in endothelial cell function/dysfunction, blood pressure control and hypertension, represents a major milestone in hypertension research. However, by this review we try to bring light on some of them in order to understand the main role of miRNAs in controlling EC

function/dysfunction and signaling pathways in response to a variety of pathophysiological stimuli.

Anywise, further investigations are required to elucidate the interactions and precise mechanisms of the involvement of miRNAs in the endothelial dysfunction and hypertension. Moreover, the inhibition or activation of miRNAs should be investigated in the future as a potential contribution of these at hypertension associated with endothelial dysfunction. Thus, the development of miRNA therapeutics could be a real challenge.

We envisage that miRNAs may be used as diagnostic biomarkers and therapeutic targets for the endothelial dysfunction in hypertensive patients.

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Compliance with Ethical Standards

Conflict of Interest Drs. Nemecz, Alexandru, Tanko, and Georgescu declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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